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# *The Molecular Biology of*

The University of Maryland

Program in Neuroscience

Fourth Annual Symposium

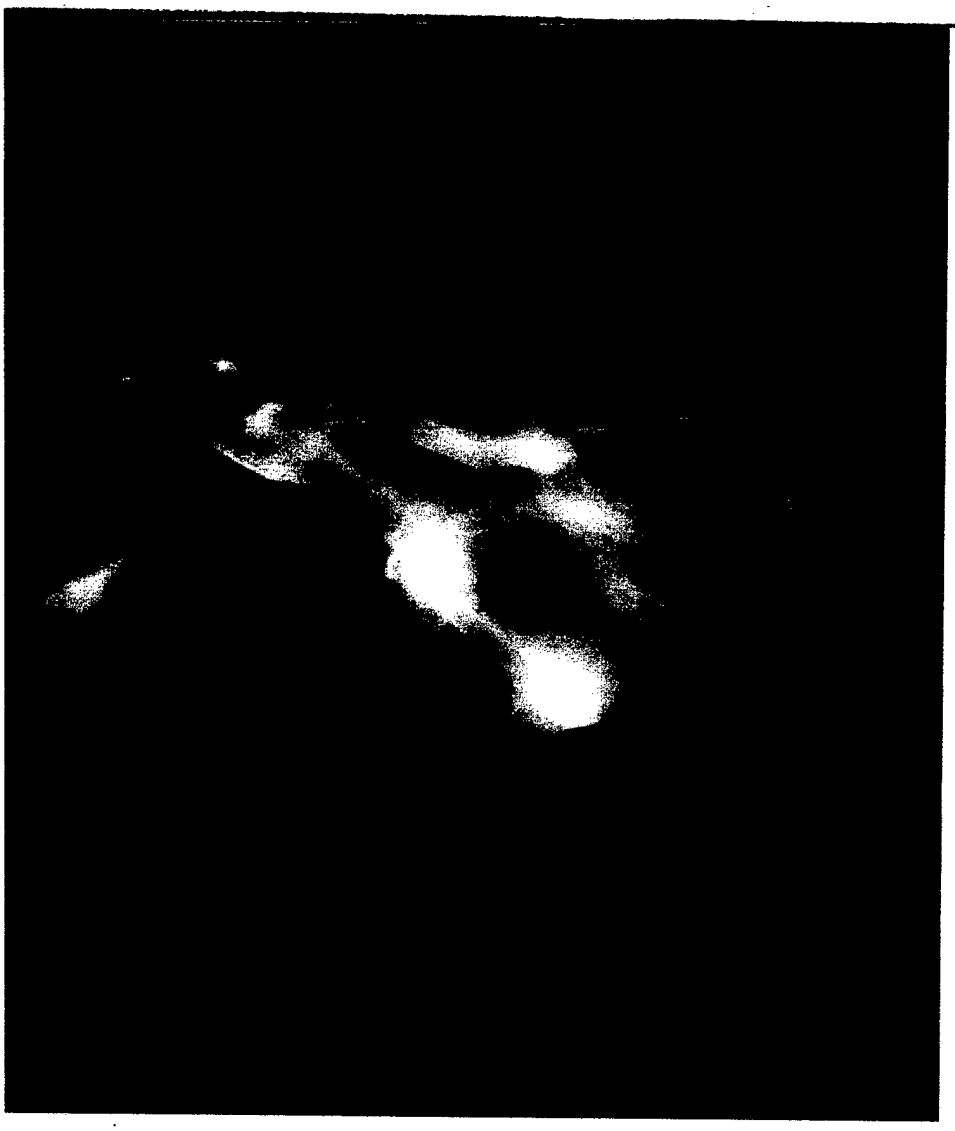
May 1, 2001

8 a.m. - 5 p.m.

School of Nursing Auditorium

655 W. Lombard St.

Baltimore, MD



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*Dear Colleagues:*

*It is a pleasure to host the Program in Neuroscience's fourth annual symposium, entitled, "The Molecular Biology of Neurodegeneration." Benefits of this symposium are twofold. It presents a unique opportunity for some of the nation's leading scientists to share their research, discuss major scientific trends, and ultimately, add to our collective knowledge of neurodegenerative diseases, as well as underscore the University's investment in excellence.*

*The University's Center for Clinical Trials facilitates the highest-quality clinical studies, such as helping Parkinson's investigators negotiate their projects from pre-study through reporting. Moreover, the October groundbreaking for the Health Sciences Facility II has set in motion a promising future for innovative studies and approaches to studying and treating diseases. We are extremely proud of these efforts and realize that the flow of knowledge among research institutions is vital in bringing solutions to these challenging and complex diseases.*

*Thank you for joining us at this year's symposium. We trust your visit to our campus proves to be enjoyable, rewarding, and encourages future collaborations.*

*Sincerely,*

*David J. Ramsay, DM, DPhil*

*President*
*University of Maryland*

NEURO

Based at the downtown Baltimore campus of the University of Maryland, home to the state's health sciences, legal, and social sciences professional schools, the Program in Neuroscience, which began in 1996, offers a doctorate in four major research areas: behavioral/systems, cellular/molecular, cognitive/computational, and developmental neuroscience. Michael T. Shipley, PhD, is director of the program and chairman of the Department of Anatomy and Neurobiology at the University of Maryland School of Medicine.

## ABOUT THE PROGRAM IN NEUROSCIENCE

SCIENCE

The Program in Neuroscience has collaborators in basic and clinical departments from several schools on campus. The program emphasizes a multidisciplinary approach to neuroscientific problems and broad training in neuroscience for graduate students and fellows. Participating faculty members occupy nearly 40,000 square feet of laboratory space, equipped with state-of-the-art facilities for all facets of contemporary neuroscientific research. The program is affiliated with the University of Maryland School of Medicine, School of Pharmacy, Dental School, the statewide Program in Neuroscience and Cognitive Sciences at the University of Maryland's Baltimore County and College Park campuses.

**8:15 a.m.**
**Welcome**

*Michael T. Shipley, PhD*
Program Director

**8:25 a.m.**
**Introduction—Neurodegenerative Disease—Shared Features and Mechanisms**

*Paul Fishman, MD, PhD*
University of Maryland Baltimore, Veterans Administration Medical Center

**Molecular Genetic Approaches**

**8:45 a.m.**
**Molecular Mechanisms of Alzheimer's Disease**
*Sangram S. Sisodia, PhD*
University of Chicago

**9:15 a.m.**
**Presinilins and Alzheimer's Disease**
*Mervyn Monteiro, PhD*
University of Maryland Baltimore

**9:45 a.m.**
**Pathobiology of Synucleinopathies**
*Virginia Lee, PhD*
University of Pennsylvania, School of Medicine

**10:15 a.m.**
**Pathogenesis of Huntington's Disease**
*Marian DiFiglia, PhD*
Massachusetts General Hospital

**10:45 a.m.**
**Break**

Fourth Annual Symposium | May 1, 2001 | 8 a.m. - 5 p.m. | School of Nursing Auditorium | 655 W. Lombard St. Baltimore, MD 21201

**Growth Factors**

**11:00 a.m.**
**Trophic Factors and Their Modulators in the Treatment of Neurodegenerative Diseases**
*Dana C. Hilt, MD*
Guilford Pharmaceuticals

**11:30 a.m.**
**Modifying the Structure and Function of the Nervous System with Genomic HSV Vectors**
*David J. Fink, MD*
University of Pittsburgh

**12:00 p.m.**
**Lunch Break**

**Mitochondria and Neurodegeneration**

**1:30 p.m.**
**Mitochondrial Molecular Targets for the Treatment of Acute Brain Injury**
*Gary Fiskum, PhD*
University of Maryland
Baltimore

**2:00 p.m.**
**Mitochrondrial Genes and Neurological Disease**
*Salvatore DiMauro, MD*
Columbia University

**2:30 p.m.**
**Mitochondria and Neurodegeneration**
*M. Flint Beal, MD*
Weil Cornell
Medical College

**Animal Models of Neurodegenerative Disease**

**3:00 p.m.**
**Defective Neurotrophin Signaling in the Trisomy 16 Mouse**
*Bruce K. Krueger, PhD*
University of Maryland
Baltimore

**3:30 p.m.**
**Role of Protein Aggregates in the Pathogenesis of Neurogenerative Disease**
*David Borchelt, PhD*
Johns Hopkins School
of Medicine

*Paul Fishman, MD, PhD*

*Dr. Fishman is professor of neurology at the University of Maryland School of Medicine, and director of the Division for Neurodegenerative Diseases.*

*He received his PhD from Yale University, his MD from The Johns Hopkins University School of Medicine, and his training in neurology was completed at Columbia-Presbyterian Medical Center. He is the founder of the Alzheimer's and Parkinson's Disease Clinic at the University of Maryland as well as the Medical Advisory Board of the Alzheimer's Association of Maryland.*

*He is the former chair of the Institutional Review Board for the supervision of human research at the University of Maryland Baltimore. His current research is supported by the Department of Veterans Affairs, where he also chairs the review group for Neurology and Neurobiology, as well as the NIH (NINDS) and the ALS Association. He has a longstanding interest in experimental therapies for Alzheimer's disease, Parkinson's disease, and ALS, with a research program devoted to the development of vectors to deliver therapeutic proteins to neurons.*

***Introduction***

## Neurodegenerative Disease—Shared Features and Mechanisms

Although Alzheimer's disease, Parkinson's disease, and ALS have widely different clinical presentations they share several aspects that suggest common basic underlying mechanisms. All are diseases of older adults, where relatively restricted groups of neurons undergo progressive dysfunction and death. All usually occur as sporadic illnesses, but a significant minority of cases have a clear familial basis. Several genes have been identified that cause inherited forms of each, almost always on an autosomal dominant basis, resulting in aberrant forms of proteins that appear to have a novel toxic gain of function. The sole exception so far is the parkin gene where loss of its ubiquitin ligase activity results in an inherited form of Parkinson's disease. Understanding the normal function of parkin may provide insight into how mechanisms such as protein processing, degradation, and oxidative injury interact to cause the selective neuronal loss of neurodegenerative diseases.

*Sangram S. Sisodia, PhD*

*Sangram S. Sisodia, is The Thomas A. Reynolds, Sr., family professor of neuroscience and chairman of the Department of Neurobiology, Pharmacology & Physiology at the University of Chicago. Dr. Sisodia earned his BA in chemistry from the College of Wooster (Ohio) in 1977 and his PhD in Biochemistry from the University of Georgia in 1985. He conducted his postdoctoral training in the Department of Biological Chemistry at The Johns Hopkins University School of Medicine under the aegis of Drs. Don Cleveland and Barbara Sollner-Webb. In 1988, Dr. Sisodia joined the Neuropathology Laboratories in the Department of Pathology at The Johns Hopkins School of Medicine as a Research Associate. In 1990, he was promoted to assistant professor of Pathology and rose to the rank of professor of Pathology and Neurosciences in 1998. In 1998, he joined the University of Chicago as chairman of the Department of Neurobiology, Pharmacology and Physiology. Dr. Sisodia has been the recipient of several awards, including the 1997 Potamkin Prize for Alzheimer's Disease Research from the American Academy of Neurology, the prestigious Metropolitan Life Foundation Award for Medical Research in 1998, and selection in 1999 as the annual medical honoree of the National Alzheimer's Association. He serves on the editorial boards of several scientific journals, including Cell and Neuron.*

**Molecular Mechanisms of Alzheimer's Disease**

Mutations in genes encoding the type I membrane protein, amyloid precursor protein (APP), or the polytopic membrane proteins, termed presenilins (PS1 and PS2) cosegregate with pedigrees with autosomal dominant, familial Alzheimer's disease (FAD). FAD-linked PS1 mutants enhance neuronal vulnerability in vivo and promote processing within the APP transmembrane domain leading to increased production of highly fibrillogenic Aß42 peptides and the acceleration of Aß deposition in brains of transgenic mice. PS1 is essential for intramembranous ("γ-secretase") processing of APP and an aspartyl residue at position 385, proposed to reside within the membrane bilayer, plays a critical role in the process. We show that a region that includes aspartate 385 does not span the membrane, and that PS1 with a mutation of aspartate 385 affects the trafficking of both full-length APP and the carboxyl-terminal APP derivatives that serve as the γ-secretase substrates. PS1 is also necessary for trafficking the BDNF receptor, TrkB, and electrophysiological studies in primary neurons indicate that loss of PS1 function has profound effects on synaptic transmission. Finally, several in vivo and in vitro studies have indicated that PS1 plays a role in the Notch proteolysis and signaling. Using a Notch-GFP chimera expressed in PS1 wt and PS1-deficient cells, we show that PS1 plays a critical role in mediating ligand- or EDTA-dependent intramembranous processing of the Notch chimera and nuclear signaling of the cytoplasmic NICD fragment. We have recently developed a cell-free assay in which γ-secretase processing of APP and Notch is readily detected and ongoing studies are aimed at the identification of PS1-associated factors responsible for "γ-secretase" activity.

*Mervyn J. Monteiro, PhD*

*Dr. Monteiro is an associate professor in the Medical Biotechnology Center and Department of Neurology, University of Maryland Biotechnology Institute and University of Maryland School of Medicine, respectively. He is a member of the Neuroscience Program and associate director of the Molecular and Cell Biology Graduate Program. He received a BSc degree in Microbiology from Queen Elizabeth College, University of London, and a PhD degree from the MRC National Institute for Medical Research, Mill Hill. He pursued postdoctoral studies in the Department of Developmental Biology at the MRC, Mill Hill. After a three-year postdoctoral fellowship with Dr. Don Cleveland at The Johns Hopkins University School of Medicine, he joined the faculty at UMB. His research in aging, with emphasis on Alzheimer's disease, has been supported by grants from the American Health Assistance Foundation and the National Institutes on Aging.*

**Presinilins and Alzheimer's Disease**

Mutations in human presenilin 1 and presenilin 2 (PS1 and PS2, respectively), two homologous genes, account for the majority (>50%) of familial Alzheimer's disease (FAD). We are studying the function of presenilin proteins and the mechanism by which FAD mutations cause disease. We have shown that overexpression of PS2 in HeLa cells leads to apoptosis and that the FAD PS2(N141I) mutation causes increased apoptosis. Moreover, we have been the first to show that presenilins are involved in cell cycle regulation, as overexpression of presenilin proteins in dividing cells leads to arrest of cells at the G1/S phase of the cell cycle. Interestingly, cell cycle arrest is potentiated by the FAD mutations in PS1 and PS2 proteins. Using the yeast 2-hybrid system we have identified several presenilin-interacting proteins. One of these is a myristoylated calcium-binding protein, which we named calmyrin (for calcium binding myristoylated protein with homology to calcineurin). Calmyrin interacts with the PS2-loop region in yeast 2-hybrid assays, in vivo colocalization of the two full-length proteins, and by increased binding of the two proteins by affinity chromatography and coimmunoprecipitation. The two proteins when coexpressed in HeLa cells induce additive cell death. Ubiquilin is a second and novel presenilin-binding protein that we identified. Yeast two-hybrid (Y2H) interaction, GST pull-down experiments, and colocalization of the proteins expressed in vivo, together with coimmunoprecipitation and cell fractionation studies, provide compelling evidence that ubiquilin interacts with both PS1 and PS2. Ubiquilin is noteworthy for its multiple ubiquitin-related domains, typically thought to be involved in targeting proteins for degradation. However, we have shown that ubiquilin promotes increased presenilin protein accumulation. Pulse-labeling experiments indicate that ubiquilin facilitates increased presenilin synthesis without substantially changing presenilin protein half-life, suggesting that ubiquilin may act as a molecular chaperone. Studies on the function of presenilin proteins and the proteins with which they interact are likely to provide important insights for the development of rational therapies for AD.

*Virginia Man-Yee Lee, MBA, PhD*

*Dr. Lee is a senior fellow at the University of Pennsylvania's Institute on Aging. Dr. Lee earned a PhD in biochemistry in 1973 at the University of California, San Francisco. In 1984, she received an MBA from the University of Pennsylvania. She is co-director of UPenn's Center of Neurodegenerative Disease Research and a professor of pathology and laboratory medicine at the School of Medicine. Her primary research interests are the neuronal cytoskeleton and amyloid beta precursor proteins and their roles in the pathobiology of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.*

## Pathobiology of Synucleinopathies

Since the identification of mutations in the α-synuclein gene in familial Parkinsons' disease (PD), α-synuclein has been implicated as a major component of the abnormal filaments that form Lewy bodies (LBs) in PD, diffuse Lewy body disease (DLB), and a Lewy body variant of Alzheimer's disease, and glial cytoplasmic inclusions (GCIs) in multiple system atrophy (MSA). These neurodegenerative diseases are collectively known as synucleinopathies. Recent studies have shown that the recombinant α-synuclein assembles into 10-nm-diameter filaments that closely resemble those found in Lewy bodies. The A53T mutation in α-synuclein increases the rate of formation, as well as the amount, of assembled filaments when compared with wild-type α-synuclein. We also identified amino acid residues 71-82 of α-synuclein as essential for filament formation. Because oxidative stress has been implicated as a pathogenic mechanism for PD, we assessed whether or not α-synuclein is a target for oxidation-induced tyrosine cross-linking and tyrosine nitration. Using a variety of approaches, we show that α-synuclein is a target for tyrosine cross-linking and tyrosine nitration in LBs and GCIs. Our data provide evidence to directly link oxidative and nitrative damage to the onset and progression of neurodegenerative synucleinopathies.

*Marian DiFiglia, PhD*

*Dr. DiFiglia is a professor in neurology at Harvard Medical School and director of the Laboratory of Cellular Neurobiology at the Massachusetts General Hospital. After graduating from Queens College in New York, she received a PhD in neuropsychology and neurophysiology in 1973 from the City University of New York. Her postgraduate studies in neuroanatomy were completed at the Mount Sinai Medical School in New York, and she was subsequently appointed to the faculty in neurology there. Since 1980, she has been a member of the Harvard Medical School faculty and has conducted research at Massachusetts General Hospital.*

## Pathogenesis of Huntington's Disease

The expansion of a polyglutamine tract in the N-terminal region of huntingtin causes dysfunction and death of striatal and cortical neurons in Huntington's disease (HD). How this occurs is unclear. A gain of function by the mutant protein has been proposed to explain pathogenesis. Polyglutamine expansion in huntingtin may change its solubility, metabolism, and/or its binding properties with interacting proteins, thereby leading to changes in function of the mutant protein in cells. Another possibility is that mutant huntingtin attenuates a function of wild-type huntingtin. The role of wild-type huntingtin in neurons is unknown. Endogenous wild-type huntingtin localizes mainly to the cytoplasm in neurons. We showed the widespread distribution of huntingtin in secretory and endocytic pathways. In brain and fibroblasts, wild-type huntingtin associates with vesicle membranes, endosomes, the trans Golgi network, and plasma membranes and appears in synaptosomal membrane fractions by Western blot. We found clathrin, a protein required for membrane budding, co-localized with huntingtin on vesicles in the cytoplasm, the trans Golgi network, and at the plasma membrane. In axons, the huntingtin associated with vesicle membranes moves anterogradely by fast transport and is retrogradely transported, consistent with a presence on endosomes. Huntingtin's association with endosomes is altered after stimulation with forskolin, which activates cyclic AMP, or by a dopamine D1 receptor agonist. This suggests that huntingtin's role in intracellular membrane trafficking is linked to receptor activation at the cell surface. Some proteins that interact with huntingtin are localized to vesicle membranes and function in membrane trafficking and cytoskeleton stability. These interactors bind differently to mutant huntingtin than to wild-type huntingtin, thereby favoring the possibility that there is altered membrane trafficking in HD.

*Dana C. Hilt, MD*

*Dr. Hilt is vice president of Clinical Research at Guilford Pharmaceuticals, Inc. in Baltimore. He received his MD from Tufts University and trained in Internal Medicine at Harvard University and Neurology at The Johns Hopkins University. After postdoctoral training at the NIH with Marshall Nirenberg in molecular neurobiology he joined the Department of Neurology, University of Maryland School of Medicine. In 1993 he was appointed director of clinical neuroscience at Amgen, Inc., in Thousand Oaks, Calif., where he was involved in the clinical trials of BDNF, NT-3, and GDNF. In 1998 he assumed his present position and is responsible for ongoing clinical development trials testing neuroprotective small molecules in a number of neurodegenerative diseases.*

**Trophic Factors and Their Modulators in the Treatment of Neurodegenerative Diseases**

Trophic factors are target-derived proteins that promote the growth, survival, and function of specific neurons. Originally described as essential factors for the development of the nervous system, trophic factors have been described recently as having the ability to promote the function and survival of adult neurons in both the normal adult nervous system and in specific neurodegenerative disease models. Both protective and restorative actions have been observed in preclinical animal models. For example, nerve growth factor (NGF) can prevent cholinergic neuronal cell death and dysfunction in models of Alzheimer's disease. Glial-cell line derived neurotrophic factor (GDNF) has similar trophic actions on dopaminergic neurons in Parkinson's disease models including the primate MPTP model. The activities of a number of neurotrophic protein growth factors in neurodegenerative disease models will be reviewed. In addition to protein growth factors, a number of small-molecule trophic factors/modulators have been developed recently. In some cases, these compounds have actions similar to neurotrophic protein factors in disease models. For example, neuroimmunophylin ligands are small molecular compounds that possess neurotrophic activities similar to those described with FK-506 on a variety of neuronal cell types, but lack the immunosuppressant actions. These agents have had significant trophic actions in Parkinson's disease models (MPTP protective and restorative rodent and primate models) and Alzheimer's disease models (aged rodent memory function and biochemical enhancement of cholinergic neuron function). The small-molecule neurotrophic factors/modulators offer the potential benefits of oral dosing and easier passage through the blood brain barrier. These problems have impaired the ability to study protein neurotrophic factors. Early clinical trials have been conducted with protein neurotrophic factors in a number of neurodegenerative diseases including Alzheimer's and Parkinson's disease, peripheral neuropathy, and amyotrophic lateral sclerosis. These clinical results will be reviewed. Another review will cover early clinical trials initiated recently with small molecule trophic factors/modulators.

*David J. Fink, MD*

*Dr. Fink is professor of neurology, molecular genetics and biochemistry at the University of Pittsburgh, chief of the Neurology Service and Director of the Geriatric Research Education and Clinical Center (GRECC) at the VA Pittsburgh Healthcare System. He received a BA from Yale College, an MD from Harvard Medical School, completed a residency in internal medicine at the Massachusetts General Hospital, a residency in neurology at the University of California San Francisco (UCSF), and trained in research with Dr. Harold Gainer in the laboratory of neurochemistry at the NIH. After 12 years at the University of Michigan, he moved to the University of Pittsburgh where he has been since 1995. Dr. Fink's research, funded by the NIH, the Department of Veterans Affairs, and several private foundation grants, is focused on the development of recombinant herpes simplex virus vectors for the treatment of neurologic conditions.*

**Modifying the Structure and Function of the Nervous System with Genomic HSV Vectors**

There are three different strategic approaches to use HSV-based vectors to treat diseases of the nervous system in rodent models: (1) direct stereotactic inoculation to specific sites in the central nervous system; (2) peripheral inoculation, using retrograde axonal transport to deliver the vector from the skin to sensory neurons of the dorsal root ganglion; and (3) infection of other tissue (e.g., fat) for the continuous production and release of the transgene product resulting in systemic delivery. A recombinant genomic HSV vector containing the coding sequence for the human anti-apoptotic peptide bcl-2 protects dopaminergic neurons of the rat substantia nigra from cell death caused by injection of the neurotoxin 6-hydroxydopamine into the striatum, and simultaneously preserves tyrosine hydroxylase expression in lesioned cells. A vector expressing glial-derived neurotrophic factor (GDNF) acts additively to improve both cell survival and neurotransmitter expression. A recombinant genomic HSV vector expressing nerve growth factor (NGF) injected subcutaneously in the foot prevents the development of sensory neuropathy caused by overdose of pyridoxine, and is also effective in preventing the development of diabetic sensory neuropathy in the mouse. An unexpected finding has been the observation that infection of "ectopic" sites, not infected in nature (such as subcutaneous fat), may result in the continuous release into the blood of at least one transgene product (NGF). In the streptozotocin-diabetes model in the mouse, inoculation of the NGF-expressing vector into fat prevents the reduction in foot sensory nerve amplitude characteristic of neuropathy in that model. By exploiting the natural neurotropism of HSV, we have constructed a series of recombinant vectors that can be used to protect neurons of the central nervous system from the degeneration induced by toxins or trauma, and which may also be used to modify the physiology of the nervous system through synthesis and release of neurotransmitters.

*Gary Fiskum, PhD*

*Dr. Fiskum is a professor and research director of anesthesiology, professor of biochemistry and molecular biology, and professor of pharmacology and experimental therapeutics. He earned his bachelor's degree from the University of California, Los Angeles and his doctorate in biochemistry from St. Louis University. As a postdoctoral fellow, he studied with Professor Albert L. Lehninger at The Johns Hopkins University. He then served on the biochemistry faculty of George Washington University for 16 years before moving to the University of Maryland Baltimore. Dr. Fiskum is a regular grant reviewer for the NIH and is the recipient of research grant awards from the NIH, the Department of Defense, and several pharmaceutical firms. He has received international recognition for his work on mitochondrial dysfunction and acute brain injury and serves as the convener of the neuroprotection research focus group at the University of Maryland Baltimore.*

## Mitochondrial Molecular Targets for the Treatment of Acute Brain Injury

Alterations in a few key parameters appear to be responsible for inducing both necrosis and apoptosis in experimental models of both acute and delayed neural cell death following stroke, cardiac arrest, head trauma. These alterations include an increase in intracellular $Ca^{2+}$, a decrease in pH, and an increase in reactive oxygen species. If the extent or the duration of these alterations is sufficiently great that cells are de-energized for prolonged periods and cellular membranes become irreversibly damaged, necrosis and secondary inflammatory tissue injury ensue. If, however, cellular ATP levels can be maintained, cells may recover or proceed through a "programmed" series of steps toward apoptotic cell death. Elevated intracellular $Ca^{2+}$ caused by massive influx through both ligand-gated channels, e.g., glutamate receptors, and voltage-gated channels, acts at the level of mitochondria to cripple their ability to respire and generate ATP and to stimulate the production of toxic reactive oxygen species. Alternatively, $Ca^{2+}$ may trigger the release of the protein cytochrome c from its normal location between the inner and outer mitochondrial membranes into the cytosol where it acts together with other factors to activate a class of proteases known as caspases that mediate the process of apoptosis. The release of cytochrome c and other apoptogenic proteins from mitochondria is also triggered by specific pro-apoptotic intracellular proteins, e.g., Bax, and inhibited by others, e.g., Bcl-2. Our work has provided insight into the mechanisms of mitochondrial dysfunction that compromise cerebral energy metabolism and promote apoptosis. This knowledge has led to the development of neuroprotective interventions, e.g., intravenous administration of acetyl-L-carnitine or cyclosporin A, and exposure to hyperbaric oxygen, that are being tested in animal models or clinical trials for ischemic or traumatic brain injury.

*Salvatore DiMauro, MD*

*Dr. DiMauro graduated from the University of Padova in Italy with a specialization in neurology in 1967. He came to the University of Pennsylvania in 1968 and began receiving MDA research grants in 1969. DiMauro has served as an MDA scientific adviser and has written hundreds of articles and book chapters on muscle disorders. Since 1991, he has been the Lucy G. Moses Professor of Neurology at Columbia University.*

**Mitochrondrial Genes and Neurological Disease**

The small, maternally inherited mitochondrial DNA (mtDNA) is a veritable Pandora's box of pathogenic mutations. Thirteen years into the era of "mitochondrial medicine," more than 100 point mutations and innumerable rearrangements (deletions, duplications, or both) have been associated with a bewildering variety of multisystemic, as well as tissue-specific, human diseases. After reviewing the principles of mitochondrial genetics, comparisons will be made between the clinical and pathological features of disorders due to mutations affecting mitochondrial protein synthesis and disorders caused by mutations in protein-coding genes. In contrast with the remarkable progress in understanding etiology, pathogenesis is not completely explained by the rules of mitochondrial genetics. For example, we do not understand why mutations in two tRNA genes, both impairing protein synthesis to similar extents, should give rise to different syndromes, MERRF and MELAS. Nor is it understood why epilepsy is invariably present in both MERRF and MELAS, but only rarely in Kearns-Sayre Syndrome, another defect of mitochondrial protein synthesis. There has been some recent progress in epidemiology and genetic counseling, but therapy is woefully inadequate. However, several therapeutic approaches are being explored and will be reviewed.

*M. Flint Beal, MD*

*Dr. M. Flint Beal is an Anne Parish Titzel professor of neurology and neuroscience and chairman of the Department of Neurology and Neuroscience at Weil Cornell Medical College in New York. Dr. Beal received his BA in 1975 from Colgate University and his MD in 1976 from the University of Virginia. Dr. Beal came to New York Weil Cornell from Harvard Medical School and Massachusetts General Hospital, where he was chief of the Neurochemistry Laboratory and director of the Clinical Trials Unit in the Department of Neurology.*

**Mitochondria and Neurodegeneration**

There is substantial evidence implicating mitochondria as playing a crucial role in both necrotic and apoptotic cell death. Mitochondria are essential in controlling specific apoptosis cell death pathways. There is increasing evidence implicating mitochondrial dysfunction in the pathogenesis of neurodegenerative diseases such as ALS and Huntington's disease. We and others have shown that lactate is elevated in the cortex of HD patients, that there is reduced phosphocreatine to inorganic phosphate ratio in resting muscle of HD patients, that the maximum rate of mitochondrial ATP generation in muscle is reduced in both symptomatic HD patients and presymptomatic gene carriers and that HD lymphoblast mitochondria show increased susceptibility to depolarization that directly correlates with CAG repeat lengths. Furthermore, mitochondrial toxins produce striatal lesions, which closely resemble the histopathology of HD. In ALS, muscle biopsies show increased mitochondrial volume and calcium levels. There is reduced cytochrome oxidase activity in the anterior horn motor neurons in patients with sporadic ALS. We have found increased mitochondrial DNA point mutations in spinal cord tissue. A study of ALS cybrids show a significant decrease in complex I activity as well as trends towards reduced complex III and IV activities. We found increased levels of 8-hydroxy-2-deoxyguanosine, a marker of oxidative damage to DNA in the plasma, urine and CSF of sporadic ALS patients. There is also evidence of mitochondrial dysfunction in transgenic mouse models of both HD and ALS. We found that administration of creatine, which may increase cellular phosphocreatine levels, can significantly improve survival and motor function in transgenic mouse models of both HD and ALS. We have also found that a number of other agents that improve mitochondrial function also show efficacy. It is, therefore, possible that therapeutic strategies aimed at improving mitochondrial dysfunction may be beneficial in the treatment of neurodegenerative diseases.

*Bruce K. Krueger, PhD*

*Dr. Krueger is a professor of physiology at the University of Maryland School of Medicine and a member of the Brain Injury and Neuroprotection focus group of the UMB Program in Neuroscience. He received his BS and PhD degrees from Yale University. After postdoctoral training at Washington University School of Medicine, he joined the faculty of the UMB School of Medicine in 1979. Dr. Krueger was the recipient of an Alfred P. Sloan Fellowship in neuroscience and was awarded Fogarty and Guggenheim fellowships for his research while on sabbatical leave with Martin Raff, University College London. His current research is supported by the National Institutes of Health (NIA, NINDS) and focuses on the molecular mechanisms underlying neurodegeneration and abnormal brain development in animal models of Down syndrome and Alzheimer's disease.*

## Defective Neurotrophin Signaling in the Trisomy 16 Mouse

Two of the hallmarks of Down syndrome (DS; trisomy 21) are mental retardation and the early occurrence of Alzheimer's disease. We have observed accelerated death (apoptosis) of neurons in vitro and in vivo in the hippocampus of the trisomy 16 (Ts16) mouse, an animal model of DS. Cultured Ts16 hippocampal neurons undergo accelerated apoptosis due to their failure to respond to the neurotrophin, BDNF, which is produced by the neurons themselves and promotes their survival. Ts16 neurons have elevated levels of the inactive, truncated isoform of the BDNF receptor, trkB, leading to the hypothesis that truncated trkB overexpression causes the BDNF signaling failure and accelerated death. Adenovirus-mediated introduction of exogenous full-length trkB into Ts16 neurons fully restored BDNF-mediated survival, whereas exogenous truncated trkB expression in normal, euploid neurons reproduced the Ts16 BDNF signaling failure. Thus, aberrant expression of trkB isoforms selectively eliminates the ability of BDNF to promote the survival of Ts16 neurons and this defect can be corrected by genetic manipulation of trkB expression. A general failure of BDNF signaling could contribute to neurological disorders not only by increasing neuron death, but also by altering the modulation of neural connectivity and synaptic plasticity by BDNF thereby compromising cognitive function.

*David Borchelt, PhD*

## Role of Protein Aggregates in the Pathogenesis of Neurogenerative Disease

*Dr. Borchelt is an associate professor of pathology and neuroscience at The Johns Hopkins University School of Medicine. He received his BS, MS, and PhD degrees from the University of Kentucky and his postdoctoral training at the University of California, San Francisco, in the laboratory of Dr. Stanley Prusiner. In 1992 Dr. Borchelt joined the faculty of Johns Hopkins. Dr. Borchelt is a cellular/molecular biologist who has worked on a variety of in vitro and in vivo models of genetic neurodegenerative diseases, including Alzheimer's disease, ALS, and Huntington's disease. His current work is supported by the National Institutes of Neurologic Disease and Stroke, National Institutes of Aging, the Huntington's Disease Society of America, and the Hereditary Disease Foundation.*

In familial Alzheimer's disease, familial amyotrophic lateral sclerosis (FALS), and Huntington's disease, mutations in specific proteins (e.g., presenilins and amyloid precursor protein in familial Alzheimer's disease, superoxide dismutase I in familial ALS, and huntingtin in Huntington's disease) create molecules that are toxic to specific populations of neurons. The mechanisms by which these mutant proteins injure and destroy subsets of neurons remains elusive. However, a common theme in each of these disorders is the extracellular, intracellular, or intranuclear accumulation of aggregated proteins in neurons and astroglial. Primary interest is to understand how these aggregates of protein lead to the dysfunction and death of selected populations of neurons. The mechanisms by which mutations in the enzyme superoxide dismutase 1 (SOD1) cause FALS have remained elusive. In the present study of mice expressing three different mutant proteins (G37R, G85R, and G93A), we demonstrate an age-dependent aggregation of SOD1 monomers into macromolecular structures that are retained after filtration through cellulose acetate. Further, we demonstrate similar aggregates of ß-amyloid peptides and tau in the brains of Alzheimer's patients. We suggest that the mechanisms of motor neuron degeneration in FALS, caused by mutations in SOD1, may overlap with those of other neurodegenerative disorders where the accumulation of aggregated protein plays a pivotal role in disease pathogenesis.


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